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Effect of season, growth regulators and simultaneous grafting on the rootability of Juniperus scopulorum and Juniperus horizontalis stem cuttings

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THE EFFECT OF SEASON, GROWTH REGULATORS AND SIMULTANEOUS GRAFTING ON THE ROOTABILITY OF *JUNIPERUS SCOPULORUM* AND *JUNIPERUS HORIZONTALIS* STEM CUTTINGS

By

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The Effect of Season, Growth Regulators and Simultaneous Grafting on the Rootability of Juniperus scopulorum and Juniperus horizontalis Stem Cuttings (26 pp.)

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The objective of this study was to develop a vegetative propagation technique for Juniperus scopulorum and Juniperus horizontalis. Stock plants were selected from natural stands in the Colstrip, Montana area. Stem cuttings of Juniperus scopulorum and Juniperus horizontalis were made in March and June of 1984. Ten rooting hormone/Captan fungicide treatments were applied in talc based dips. Cuttings of J. scopulorum were side grafted to cuttings of J. horizontalis in a effort to promote rooting of J. scopulorum. The number of rooted cuttings and the number of roots per cutting (>2mm. at the stem) were counted after 12 weeks in the mist chamber. The number of cuttings rooted for J. horizontalis stem cuttings was greater for cuttings made in June than in March (60% vs. 49.5%). However, March cuttings had a greater number of roots per cutting (6.5 vs. 4.0). The application of auxin and Captan had little effect on rooting percentage and root quality. While rooting of J. scopulorum cuttings and J. scopulorum * J. horizontalis grafts was poor (<10%), auxin was required to initiate rooting of J. scopulorum.

Acknowledgements

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INTRODUCTION

Federal and Montana state laws require that surface mined sites be revegetated with native species in order to be classified as reclaimed land. Natural forests near Colstrip, Montana are dominated by ponderosa pine (Pinus ponderosa Laws. var. scopulorum) with lesser amounts of Rocky Mountain juniper (Juniperus scopulorum Sarg.) and creeping juniper (Juniperus horizontalis Moech.). Ponderosa pine can be established through the use of containerized seedlings (Baumbauer and Blake 1984). However, juniper seed with its double dormancy requires approximately five years to produce a plantable seedling (USDA 1974).

Vegetative propagation may be an alternative which would save time and money, if a stem cutting technique for eastern Montana varieties of Rocky Mountain juniper and creeping juniper could be developed. Work with juniper cuttings appears to have been limited to ornamental types from nursery raised stock plants. Consequently, the objective of the study was to investigate the feasibility of propagating Rocky Mountain juniper and creeping juniper from stem cuttings taken from native wild plants. Specifically, the objectives of the study were to determine if:

1. Time of year when scion was collected had any effect on quantity rooted.

2. Time of year when scion was collected had any effect on number of roots per cutting (rooting quality).

3. Determine the effects of auxins and fungicide on the rooting of cuttings.

4. Determine if grafting cuttings of Rocky Mountain juniper on creeping juniper improved Rocky Mountain juniper rootability.

LITERATURE REVIEW

Species

Junipers exhibit extremely variable rooting behavior, depending on species (Fordham and Spraker 1977). Standing or vertical junipers (e.g. J. scopulorum) are difficult to root, while spreading, prostrate types (e.g. J. horizontalis) are easily rooted (Chong 1981, Hartman and Kester 1975).

Physiological Condition and Age of Parent Plant

Environmental conditions influence the endogenous levels of auxins, rooting co-factors, and carbohydrate in plants. Lanphear and Meahl (1966) and Richardson et al (1979) found that cuttings from greenhouse-grown plants rooted better than field-collected native wild plants.

The physiological condition of the stock plant also varies with the season of the year and as the plant ages. In difficult to root species, stem cuttings taken from young seedlings (those in juvenile growth phase) will root much more readily than cuttings obtained from older plants (Fordham and Spraker 1977, Hartman and Kester 1975). This is probably because as plants grow older auxin cofactors decrease and root inhibitors increase (Hartman and Kester 1975, Weaver 1972). Fordham and Spraker (1977), Hartman and Kester (1975) reported cuttings taken from

lower branches rooted better than those taken from the upper crown. They implied that lower branches apparently retain juvenile physiological characteristics.

Season

Wyman (1930) reported that more roots are produced when juniper cuttings are taken from late fall to late winter. Lanphear and Meahl (1963) found J. horizontalis cuttings had high rooting percentages (90%) and root numbers (25-30 per cutting) when taken from November through April with a peak in July. This seasonal difference was not altered by the use of root-promoting substances or by altering the environment of the cutting. Lanphear and Meahl (1966) reported that high rooting percentages during fall and winter were independent of low temperatures, implying that physiological changes such as seasonal changes in endogenous root promoting substances could result in rooting variation. Summer cuttings of J. horizontalis can be rooted if cuttings are made from shoots that include a heel of the previous year's growth (Fordham and Spraker 1977).

Growth Regulators (auxins and Captan)

The synthetic auxin, indolebutyric acid (IBA) is the most successful root promoting hormone in use today. IBA mimics the actions of naturally occurring indoleacetic acid (IAA). IAA is difficult to use since it breaks down with exposure to sunlight and has a limited shelf life. Of the various methods that are used to apply auxins, talc based

dips are the most convenient and stable (Whitcomb 1978). Table one summarizes the auxin types and concentrations used by various authors. Naphthalenacetic acid (NAA) is a stronger synthetic auxin than IBA, and has a synergistic effect on rooting of cuttings when used with IBA (Hitchcock and Zimmerman 1940).

Fungicides can improve the quality of rooted cuttings by protecting the emerging roots from fungal attack. Captan works well for treating cuttings, since it does not decompose easily and has a long residual action (Hartman and Kester 1975).

TABLE 1 - SUMMARY OF GROWTH REGULATORS USED ON JUNIPER

Author	Species	Treatment	% rooted
Lanphear(1961)	J.horizontalis	0.3% IBA	94.4
	J.chinensis	0.3% IBA	87.5
Wells (1961)	Juniper spp.	2.0% IBA + 0.4% NAA + Fungicide	N/A
Fordham (1977)	J.horizontalis	0.3-0.8% IBA	N/A
Whitcomb (1977)	J.horizontalis	0.8% IBA	N/A
Gil-Albert(1978)	J.scopulorum	0.1-0.8% IBA	0
	J.horizontalis	0.1-0.4% IBA	10-100
Chong (1981)	J.virginiana * J.sabina grafts	0.3% IBA	46-75

Environmental Conditons During Rooting

When propagating slow rooting plants, environmental conditions such as water relations, rooting medium, and air and medium temperature greatly influence rooting of cuttings.

In slow-to-root species, water loss from the leaves must be reduced to a low level to keep the cuttings alive until roots form. Intermittent mist propagation places a film of water on leaves, raising relative humidity and lowering air and leaf temperatures - all factors which lower transpiration and respiration rates (Fordham and Spraker

1977, Hartman and Kester 1975, Whitcomb 1978).

An equal volume mixture of sand, perlite, and peat was recommended as a rooting medium for conifers by Hartman and Kester (1975), Fordham and Spraker(1977), and Whitcomb (1978). Teuscher (1962) recommended using perlite as a medium for simultaneous grafting and rooting of junipers.

Simultaneous Grafting and Rooting

Simultaneous grafting and rooting involves grafting a slow-to-root scion to an unrooted, but easy-to-root cutting. Teusher (1962) reported that quicker rooting can be obtained by side-grafting scions of slow rooting *J. scopulorum* to unrooted cuttings of *J. chinensis* 'Hetzi'. Chong (1981) obtained 57% successful grafts and rooting by side-grafting *J. virginiana* 'Skyrocket'(scion) and *J. sabina* 'Blue Danube' (rootstock).

METHODS

Mist Chamber

A portable mist chamber was constructed in the School of Forestry greenhouse. It was a wooden framed structure (3'*4'*10'), with a polyethylene cover. Lead heating cables lined the floor and were covered with perlite to provide a warm, uniform base with good drainage. Two timers controlled the mist unit: a clock timer turned the system on in the morning and off at night, and an electronic timer controlled the mist frequency and duration. Two atomizer nozzles, and a sprinkler nozzle provided the mist. Frequency and duration of mist were varied with the light intensity and air temperature within the mist chamber. Typical settings for late winter-early spring period were five seconds of mist every half hour, while mid-summer settings were eight seconds of mist every five minutes.

Pilot Study

J. scopulorum cuttings were collected in May, July, August and October. Table two summarizies the auxin combinations applied. For J. scopulorum, rooting was poor (< 5% overall), but the stronger auxin concentrations appeared to effect rooting. J. horizontalis cuttings were collected in October. Rooting percentage increased, 15% overall, with 34% rooting when treated with the 2.0% IBA + 0.2% NAA + 25% Captan dip.

TABLE 2- AUXIN CONCENTRATIONS FOR PILOT STUDY.

Collection Period	Species	Growth Regulator (Talc based dip)
May & July	Rocky Mountain	0.3% IBA
		0.8% IBA
		0.3% NAA
		0.8% NAA
		0.25% IBA + 0.25% NAA
August	Rocky Mountain	1.0% IBA
		2.0% IBA
		1.0% IBA + 0.2% NAA
October	Creeping juniper	1.0% IBA
	Rocky Mountain	1.0% IBA + 0.2% NAA
		2.0% IBA
		2.0% IBA + 25% Captan
		2.0% IBA + 0.2% NAA
		2% IBA +.2% NAA + Cap.

The Study

Two collections were made, the first during 13-14 March and the second during 18-19 June 1984. Three hundred four to six-inch (10 - 15 cm) cuttings from trees of both species were taken from natural stands in the Colstrip, Montana area. Only the youngest trees, lowest branches, and current to two year old wood from lateral and terminal shoots was used for cuttings. Different stock plants were used for each collection. The cuttings were stored in plastic bags and placed on ice in a cooler during transportation and processing (one to four days).

Twenty cuttings of each species were dipped in each of the ten concentrations of growth regulator treatments (Table 3). The cuttings were inserted into 4"* 14"* 20" plastic trays containing an equal volume mixture of sand, perlite, and peat. The treatments were randomly

distributed among the trays, and the trays were rotated in the mist chamber to average any environmental influences. The cuttings were placed under a light, intermittent mist with a natural photoperiod [1]. Air temperature was maintained between 60-80 degrees F (15-27 degrees C) with bottom heat at 75 degrees F (24 degrees C).

The remaining 100 cuttings of each species were side-grafted together. Two inches from the base of the J. horizontalis cutting a downward incision 1.5 inches long was made through the bark and cambium and into the wood. The scion was prepared by making two slices on the J. scopulorum cutting to form a wedge. The cambium layers of the J. horizontalis and J. scopulorum were matched up during insertion. The grafts were held together with a rubber grafting band, and auxin/Captan treatments were applied. The grafts were placed in trays deep enough to cover the graft union and placed in the mist chamber. The grafts were examined at six weeks, and grafting bands removed.

[1] Natural photoperiod in Missoula, MT: March 15 - 12 hours
June 15 - 18 hours
Sept. 15 - 12.5 hours

TABLE 3 - GROWTH REGULATOR TREATMENTS (TALC BASED DIPS)

1. 1.6% IBA (Hormex 16)
2. 1.6% IBA + 25% Captan
3. 4.5% IBA (Hormex 45)
4. 4.5% IBA + 25% Captan
5. 1.6% IBA + .4% NAA
6. 1.6% IBA + .4% NAA + 25% Captan
7. 4.5% IBA + .4% NAA
8. 4.5% IBA + .4% NAA + 25% Captan
9. control auxin + control Captan
10. control auxin + 25% Captan

Statistical Analysis

All cuttings and grafts were examined at twelve weeks. Quantity rooted and rooting quality were described using percentage of cuttings rooted and number of roots per cutting, respectively. The number of roots greater than 2 mm long were counted for five randomly selected cuttings per treatment of J.horizontalis and ten cuttings and ten grafts per treatment of J. scopulorum. The data were transformed since it was in interval form. These data were analyzed with an analysis of variance (ANOVA) with factorial arrangement of the treatments. Table four contains ANOVA tables for the J. horizontalis data. Treatments containing auxin (1-8 in table 3) were pooled together and analyzed for both percent rooted and rooting quality data. The treatments which

included Captan (2,4,6,8,10 from table 3) were pooled together and tested. Table five contains the ANOVA table used to analyze the J. scopulorum data. The rooting quality data were not analyzed due to the lack of roots. Significance was tested at 0.05.

Transplanting and Outplanting

Successfully rooted cuttings and grafts were transplanted into 60 cc containers. The March-June cuttings and grafts were scheduled for outplanting in late October 1984. This allowed four months of root system development. The June-September rooted cuttings and grafts are to overwinter in the greenhouse and be outplanted in October 1985. The large containers will allow for root system expansion without becoming pot bound.

Thirty-five Rocky Mountain juniper rooted cuttings, five rooted grafts, and 100 rooted creeping juniper cuttings were auger planted at a two*two foot (60cm*60cm) spacing on reclaimed soils at Colstrip, MT on October 20, 1984. Survival studies will be conducted in May and October to follow winter and summer mortality. The cutting-established plantation is located next to a seedling established plantation so survival of the two propagation methods can be compared.

TABLE 4 - ANOVA TABLES FOR CREEPING JUNIPER

% rooted data			# roots/rooted cutting data		
Source	df	F	Source	df	F
Season (S)	1	4.12	Season (S)	1	7.41*
Growth Regulator Treatment (GR)	9	1.62	Growth Regulator Treatment (GR)	9	1.97
@ Error	9		S * GR	9	1.71
			Error	37	
Total	19		Total	56	

@ interaction term serves as error term since there was no replication.

Source	df	F	Source	df	F
Season (S)	1	2.88	Season (S)	1	6.03*
Auxin/control (A)	1	< 1	Auxin/control (A)	1	4.10*
S * A	1	< 1	S * A	1	< 1
Error	16		Error	53	
Total	19		Total	56	

Source	df	F	Source	df	F
Season (S)	1	3.37	Season (S)	1	5.65
Captan/Control (C)	1	3.31	Captan/Control (C)	1	< 1
S * C	1	< 1	S * C	1	< 1
Error	16		Error	53	
Total	19		Total	56	

* indicates significance at 0.05 level

Table 5 - ANOVA table for Rocky Mountain juniper

Source	df	F
Season (S)	1	1.44
Growth Reg. (GR)	9	1.32
Cutting Tech. (C)	1	< 1
S * GR	9	1.54
S * C	1	< 1
GR * C	9	1.38
@ Error	9	
Total	39	

@ Three way interaction term serves as error term due to lack of replication.

RESULTS AND DISCUSSION

Juniperus horizontalis

A summary of results appears in table six. The calculated F values for the various comparisons are listed in table four.

There was a seasonal difference in root quality. Cuttings from the March collection averaged 6.48 roots per rooted cutting, while June cuttings averaged 3.97 roots. While not significantly different at the 0.05% level, there appears to be a seasonal difference in quantity rooted. June's collection showed a higher percentage rooted than March's collection. While none of the individual auxin treatments were significantly different, the addition of auxin seemed to increase the root quality. The application of Captan appeared to have no influence on root quality or quantity rooted.

TABLE 6 - SUMMARY OF J. horizontalis RESULTS

	March - June		June - September	
	% rooted	ave. # roots/ cutting	% rooted	ave. # roots/ cutting
OVERALL	49.5	6.48 * (0.91)	60.0	3.97 * (0.67)
AUXIN	48.1	7.05 *a (0.90)	60.6	4.40 *c (0.68)
CONTROL	55.0	4.67 *b (0.90)	57.5	2.43 *d (0.49)
CAPTAN	44.0	6.45 (1.16)	55.0	3.67 (0.66)
CONTROL	55.0	6.50 (0.70)	65.0	4.24 (0.69)

* indicates statistical significance within a row.
a,b,c,d indicates significance within a column.
( ) standard deviation of transformed data.

These results correspond with Lanphear and Meahl's three studies (1961, 1963, 1966) involving J.horizontalis "Plumosa" with the exception of the present study's high rooting percentage for auxin treated summer cuttings. In the 1963 study Lanphear and Meahl obtained only 5-10% rooting for auxin treated cuttings, while 70% of the control cuttings rooted. A possible explanation for the increased rooting of June cuttings would be the physiological condition of the stock plants at the time of collection. The climate was favorable with above normal precipitation and mild temperatures (Schwarzkoph, pers.comm.), which

could allow for stock plants under little physiological stress.

Figures one and two illustrate a random selection of cuttings for an auxin treatment and control for each collection period. While no quantitative measure was taken, auxin seems to inhibit the development of secondary roots. The auxin treatments seem to encourage a more robust primary root system, while the control cuttings develop a more fibrous root system. Only after outplanting trials are conducted can any conclusion be drawn on the effect of early root system development on field survival. When March cuttings were outplanted in October of 1984, most cuttings had developed a fibrous root system (figure 3).

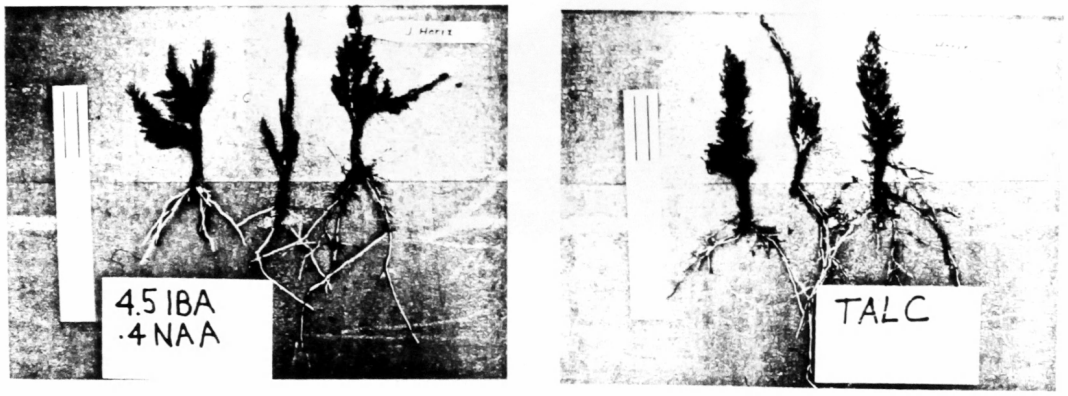

Figure 1. March - Auxin treated on left, control on right.

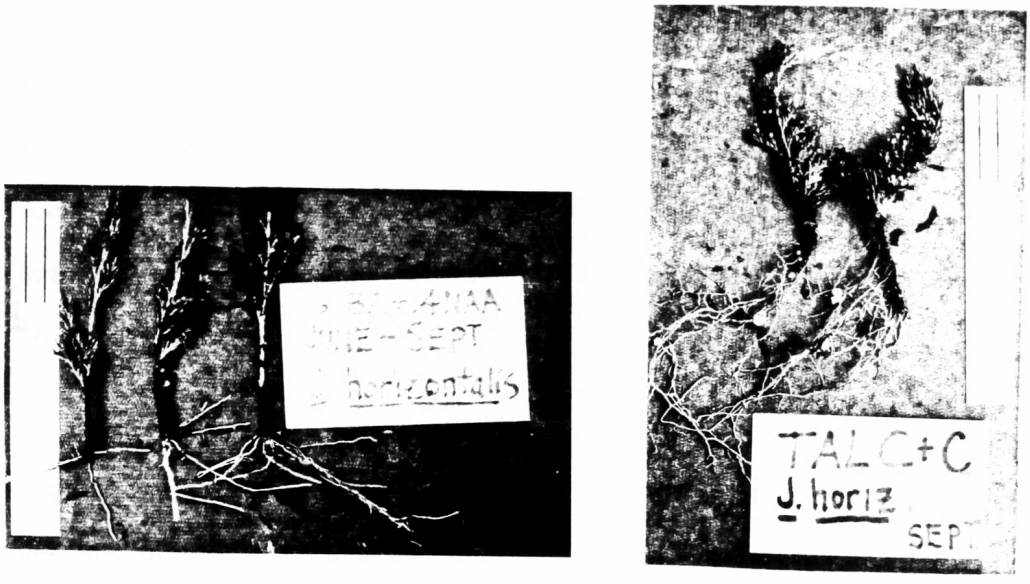

Figure 2. June - Auxin treated on left, control on right.

Figure 3. Root system development at time of outplanting.

Juniperus scopulorum

Table seven is a summary of results for J.scopulorum stem cuttings and J.scopulorum * J. horizontalis grafts. Calculated F values for the various comparisons are listed in table four. While rooting success was poor, cuttings from the March collection did appear to have greater root quality and rooting percentages. Auxin was required to initiate rooting in J. scopulorum cuttings.

The difficulty with simultaneous grafting and rooting is obtaining a graft union. Only eleven percent of the March grafts, and eight percent of the June grafts took. The tissue used for grafting was of extremely small diameter and it was difficult to match the cambium layers. A successful graft that rooted is shown in figure four.

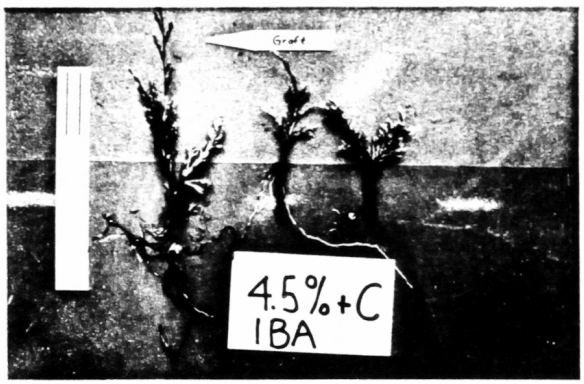

Figure 4 - Rooted graft.

TABLE 7 - SUMMARY OF *J. scopulorum* RESULTS

	March-June		June-September	
	% rooted	ave. # roots/rt. cutting	% rooted	# ave. roots/rt. cutting
OVERALL	7.0	5.57	4.0	3.00
STEM CUTTINGS	6.0	4.40	3.5	3.00
GRAFTS	9.0	6.22	5.0	3.00
AUXIN				
OVERALL	8.8	5.57	4.6	3.00
STEM CUTTINGS	6.9	4.40	4.0	3.00
GRAFTS	11.2	6.22	5.0	3.00
CONTROL				
OVERALL	0	0	1.7	3.00
STEM CUTTINGS	0	0	0	0
GRAFTS	0	0	5.0	3.00
CAPTAN				
OVERALL	8.7	6.43	2.7	3.67
STEM CUTTINGS	10.0	5.25	3.0	5.00
GRAFTS	6.0	8.00	2.0	1.00
CONTROL				
OVERALL	5.3	4.71	6.0	2.67
STEM CUTTINGS	2.0	1.00	4.0	1.00
GRAFTS	12.0	5.33	8.0	5.50

CONCLUSIONS AND SUMMARY

Juniperus horizontalis

Season

June collected cuttings appeared to have higher rooting percentages than March collection. March cuttings had greater root quality. Future collections should be made in late spring to early summer. Cuttings made during this period should be in good physiological condition, having recovered from winter- induced stress.

Growth Regulators

Auxin increased the root quality of cuttings from both collections. Future cuttings should be treated with a 1.0% - 2.0% IBA talc based dip. Captan did not appear to influence root quanity or quality. Weekly waterings with Captan could be used to discourage fungal infections.

Juniperus scopulorum

Season and Growth Regulator Influence

March cuttings and grafts appeared to have slightly higher rooting percentages and root quality than June cuttings. Auxin was required to promote rooting.

Stock Plants

Due to slow seedling growth, young wild stock plants (< 10 years old) yield very few cuttings. Future cuttings could be made from greenhouse raised seedlings. A small sample trial during the pilot study in October 1983 yielded a 25% rooting success when cuttings were made from 3-0 bareroot Rocky Mountain juniper. Alternately, rooted cuttings from the present study could be reserved to serve as stock plants for future collections. Rooting success should improve since these new stock plants represent rootable genotypes.

Simultaneous grafting and rooting

Success with simultaneous grafting and rooting was poor due to the difficulty of grafting small diameter material. Chong (1981) described a new procedure using a styrofoam block to hold the graft together. This procedure was reported to increase callus formation and strenghten the graft union. While not increasing the rooting of the two species of juniper Chong worked with. The styroblock technique might improve the success of J. scopulorum * J. horizontalis grafts.

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